the two proteins showed little interaction in the rest of the cell. Addition of a competitor peptide fully disrupted GKAP-DLC2 oligomers, leading to the inhibition of NMDA receptors activity. Taken together, these results support the notion of a hub function for DLC2 in ordering GKAP-containing complexes in dendritic spines to control glutamatergic transmission.

2953-Pos Board B108

Using Calcitriol Conjugated Quantum Dots to Target Inflammatory Breast Cancer Tumors and Metastasis In Vivo

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Calcitriol is the active form of Vitamin D_3. Epidemiological data show that women with Vitamin D deficiency at the time of breast cancer diagnosis are 94% more likely to experience cancer spread and 73% more likely to die over the next 10 years, compared to women with adequate Vitamin D levels. Since vitamin D deficiency is especially common in African American and obese women, this observation may partially explain the relatively poor clinical outcome experienced by these patients [2]. Although current treatments for IBC are very aggressive and include surgery and radiation, IBC is still the most deadly breast cancer and has a survival rate of only 40% past 5 years. Novel treatments are desperately needed [3]. Current limitations for the use of calcitriol as a treatment for IBC and non-IBC breast cancer is that high concentrations of calcitriol must be delivered to the tumor. This is even more complicated for IBC, since the tumor rapidly metastasizes and disseminates trough the lymphatic system. We successfully designed Mucin-1 (MUC-1) antibody-calcitriol conjugated Quantum Dots (MC-QDs) that infiltrate the lymphatic system and also accumulate at the original and distant tumor sites. using this approach we analyzed the distribution and accumulation of MC-QDs in vivo in an inflammatory breast cancer mouse model over 4 days using an IVES Lumina system. After 4 days, organs were extracted and accumulation of nanoparticles was analyzed. using quantitative image analysis we showed that the MC-DS accumulate at the tumor site as well as at the metastasized organs and tissues. The obtained data suggest that quantum dots can be used to image drug-tumor interactions in vivo and to deliver therapeutics to the tumor and metastasized sites as well.

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Direct, Single Molecule Observation of Early Lymphocyte Signaling

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T cells show a remarkable ability to quickly (less than 10 seconds) respond to fewer than 10 agonist molecules, even when structurally-similar self peptides outnumber agonist peptides by at least 100:1. We recapitulate these phenomena using a highly controlled supported lipid bilayer-live cell junction. using this approach we report direct, single molecule observations of T cell receptor-peptide-MHC binding kinetics coupled with stochastic simulations in order to test serial receptor triggering models within the context of our studies of membrane-proximal signal amplification in living T cells.

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Single-Molecule Fluorescence and FRET Measurements on Internalized Proteins in Living Bacteria

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Despite advances in structural biology techniques (such as NMR and X-ray crystallography), it is still extremely difficult to recover information on protein structure, conformations and dynamics inside living cells. Fluorescent protein fusions provide an avenue to study proteins in vivo but cannot directly report on protein structure due to limitations in size, labeling position, and photophysical properties; as a result, there is a pressing need for new methods that can study protein structure in vivo.

Towards this goal, we developed electroporation methods to internalize proteins labeled with organic fluorophores in living E. coli. Electroporated cells retained viability while being loaded with different singly or doubly labeled proteins of various sizes (15-100 kDa) and observed at the single-cell and single-molecule levels; single-color fluorescence or FRET could be observed for several seconds. The number of internalized proteins could be tuned from a few to several hundred copies per cell.

We used the diffusion behavior of internalized DNA-binding proteins to study protein activity and cellular localization. Internalized catabolite activator protein (a bacterial activator) remains immobile in cells, likely due to binding to chromosomal sites. In contrast, internalized DNA polymerase I Klenow fragment (KF), a component of DNA repair pathways, diffuses through the cytoplasm and localizes when DNA damaging reagents are added. Measurements on doubly-labeled KF molecules reveal FRET efficiencies centered ~50% (corresponding to an interprobe distance of ~5 nm), matching published in vitro results, and enabling the study of KF conformations and dynamics in vivo. Ongoing work focuses on internalization of partners of specific complexes to examine their structure and interactions in the bacterial cytoplasm, as well as internalization in other organisms.

2956-Pos Board B111

Insights into the Organization of an ECF-Type Transporter by Fluorescence Lifetime and Anisotropy Microscopy on Living E. Coli

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BioMNY (Rhodobacter capsulatus) is an ATP dependent, ECF-type biotin importer, consisting of two different transmembrane domains BioY, BioN and ATPase cassettes BioM. The organization and stoichiometry of the transporter subunits is still a matter of debate (Erkens, 2011; Berntsson, 2012; Erkens, 2012).

To obtain insights into the stoichiometry of the transporter components we used spectrometric, lifetime-based HeteroFRET (Finkenwirth, 2010) and static anisotropy-based HomoFRET (Kirsch, 2012) approaches. To this aim, certain transporter components, tagged with a fluorescence donor or acceptor, were co-expressed in recombinant E. coli. The measurements led to the postulation of a BioM2N?Y2 stoichiometry in living bacteria (in accordance with Neubauer, 2009, 2011).

Recently, we expanded the Hetero and HomoFRET approach form the spectroscopic to the microscopic level (Ziomkowska, 2012). The use of imaging techniques for microbial cells has the same advantages as for mammalian cells: expression level and localization of the protein can be validated in a single cell during the measurement. Our experiments show that the spectroscopic data can be reproduced in imaging experiments and will be used for the ongoing research. Nevertheless, the orientation of the probe has to be taken into account for anisotropy microscopy. To the best of our knowledge, we present the first FRET-Imaging approach on living bacteria, a tool likely to be used for interaction studies in any E. coli expression system.

2957-Pos Board B112

Single-Molecule Investigation of the Intraflagellar Transport of Membrane Signaling Proteins

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In the past decade, cilia/flagella have come to be known as essential sensory organelles for cells. These cilia/flagella perform their sensory function via signaling pathways that include flagellar membrane signaling proteins and intraflagellar transport (IFT) machinery comprised of IFT particles and BBSome particles. While the membrane signaling proteins are responsible for collecting information regarding a cell's environment, the IFT machinery has been implicated in effecting the delivery of this information through the translocation and proper positioning of the signaling proteins along the flagellar body. Unfortunately, current understanding of this intraflagellar translocation and positioning is limited to rough measurements of average IFT speeds with little regard for speed fluctuations and the causes thereof. Further, the equally important diffusive motion of signaling proteins in the flagellar membrane has been largely ignored. As such, we use single-molecule fluorescence imaging methods to study the intraflagellar motion of BBS4-GFP, IFT20-GFP, and Pkd2-GFP, a transmembrane signaling protein, in *Chlamydomonas reinhardtii*. (i) In the flagellar entry region, we have found that Pkd2 largely performs Brownian diffusion, implicating lateral membrane diffusion as a primary flagellar entry mechanism. (ii) In the flagellar body, we have found that IFT trains exhibit speed changes of approximately $\pm$ 400 nm/s, which we interpret as events of dropping off or picking up membrane signaling proteins. Futhermore, we have observed the average speed of an IFT train with a BBSome attached to be 300 nm/s slower than the speed of a train without a BBSome, indicating that the BBSome carries at least one membrane signaling protein. (iii) Finally, at the flagellar tip, BBSomes remain bound to their associated membrane signaling proteins, diffusing in the membrane at the tip for an average of two seconds before undergoing retrograde IFT.